AMENDMENT TO THE SPECIFICATION

Kindly amend the specification at page 3, line 20, through page 6, line 18, as follows:

Accordingly, in a first aspect, the invention features a molecule having a nucleic acid sequence of from 48 to 1770 nucleotides, and substantially identical to a corresponding nucleic acid sequence of SEQ ID NO. 2, that encodes a fragment of the peptide corresponding to SEQ ID NO. 1, or a homolog thereof, wherein the fragment or homolog enhances TNF- α transcription by interacting with a TNF-α promoter nucleotide sequence. Preferably, the nucleic acid sequence encodes the LSQTWREPGAAGSPFHL (SEQ ID NO: 3) peptide sequence.

In another aspect, the invention features a molecule having a nucleic acid sequence encoding the LSQTWREPGAAGSPFHL (SEQ ID NO: 3) peptide sequence, or a homolog thereof. In an embodiment of any of the nucleic acid sequences or expression constructs of the present invention, the sequence encodes a LITAF DNA binding domain for the hTNF-α promoter region, preferably nucleotides CTCCC (–515 to –511).

In another aspect, the invention features a vector that includes a molecule having a nucleic acid sequence of the invention, i.e., those encoding a fragment of the peptide corresponding to SEQ ID NO. 1, or a homolog thereof, as described above, or those encoding a LSQTWREPGAAGSPFHL (SEQ ID NO: 3) peptide sequence. Preferably, the vector is a viral vector, and most preferably is selected from the group consisting of adenoviral vectors, adeno-associated virus (AAV) vectors, retroviral vectors, hybrid adenovirus-AAV vectors, and herpes-simplex virus (HSV) vectors. The invention also features an expression construct that contains a

nucleic acid sequence encoding a fragment of the peptide corresponding to SEQ ID NO. 1 or a host cell containing this nucleic acid sequence.

In another aspect, the invention features a peptide fragment of the peptide SEQ ID NO. 1, whereby said fragment enhances TNF-α transcription by interacting with a TNF- α promoter nucleotide sequence, preferably hTNF-α. In one example, the peptide fragment includes the SQTWREPGAAGSPFHL sequence (SEQ ID NO: 5). In another example, the peptide is SQTWREPGAAGSPFHL (SEQ ID NO: 5), or a homolog thereof. In other examples, the peptide fragment includes an allelic variant of the SQTWREPGAAGSPFHL sequence (SEQ ID NO: 5) or a variant that contains a conservative amino acid substitution for a residue of this sequence, where the variant retains the ability to interact with a TNF- α promoter nucleotide sequence. These variants can include those peptides that are the result of C-terminal, N-terminal, both N- and C-terminal, or interior deletions of the full-length LITAF peptide sequence.

In another aspect, the invention features a method of identifying compounds that inhibit LITAF binding to a TNF-α promoter region that includes: a) incubating a mixture of a molecule containing a LSQTWREPGAAGSPFHL peptide sequence (SEQ ID NO: 3)(component 1); a molecule that includes a TNF-α promoter region (component 2), and the test compound (component 3); b) measuring the extent of binding of component 1 to component 2 in the absence of component 3; measuring the extent of binding of component 1 to component 2 in the presence of component 3); and determining the ratio of the binding measured in step c) to that measured in step b), wherein a decrease of binding in step c) relative to step b) indicates that the test compound inhibits the binding of LITAF to the TNF-α promoter region ion.

In another aspect, the invention features a method of identifying compounds that enhance LITAF binding to a TNF-α promoter region that includes: a) incubating a mixture of a molecule containing a LSQTWREPGAAGSPFHL peptide sequence (SEQ ID NO: 3)(component 1), wherein said molecule is not full-length LITAF; a molecule that includes the TNF-α promoter region (component 2), and the test compound (component 3); b) measuring the extent of binding of component 1 to component 2 in the absence of component 3; measuring the extent of binding of component 1 to component 2 in the presence of component 3); and determining the ratio of the binding measured in step c) to that measured in step b), wherein an increase of binding in step c) relative to step b) indicates that the test compound enhances the binding of LITAF to the TNF-α promoter region ion.

In a preferred embodiment of any of the compound-identifying methods of the invention, the peptide containing the SQTWREPGAAGSPFHL sequence (SEQ ID NO: 5) and the nucleic acid having the promoter region are not brought into contact with each other in step a) before exposing one of these components to the test compound. In another preferred embodiment, the molecule containing the LSQTWREPGAAGSPFHL sequence (SEQ ID NO: 3) is not full-length LITAF. In yet another embodiment, the TNF-α promoter region haiving a CTCCC nucleic acid sequence (SEQ ID NO: 4).

In another aspect, the invention features an antibody which binds to a LITAF fragment that contains the LSQTWREPGAAGSPFHL peptide sequence (SEQ ID NO: 3).

In another aspect, the invention features a method of suppressing tumor cell growth in an animal that includes administering a vector that includes a nucleic acid sequence of the invention, i.e., those encoding a fragment of the peptide corresponding to SEQ ID NO. 1, or a

homolog thereof, or those encoding a LSQTWREPGAAGSPFHL peptide sequence (SEQ ID NO: 3). Preferably, the vector is a viral vector, and most preferably is selected from the group consisting of adenoviral vectors, adeno-associated virus (AAV) vectors, retroviral vectors, hybrid adenovirus-AAV vectors, and herpes-simplex virus (HSV) vectors. In one embodiment, the tumor is a solid tumor, preferably of a cancer selected from the group consisting of non-small cell lung carcinoma, prostate carcinoma, renal carcinoma, colon carcinoma, ovarian carcinoma, pancreatic carcinoma and melanoma. In another embodiment, the method further includes determining if the tumor cell is deficient in p53.

Kindly amend the specification at page 10, lines 17-18, as follows:

FIG. 3 depicts the sequence of TNF-α promoter DNA from nt –550 to –487 (SEQ ID NO: 47). Note that the hLITAF-binding site is indicated by a dotted line along the top of the sequence.

Kindly amend the specification at page 18, lines 8-18, as follows:

Isolated LITAF peptide fragments can be produced recombinantly from the corresponding fragment of the nucleic acid encoding such peptides or, alternatively, can be chemically synthesized using techniques known in the art such as conventional solid phase Fmoc or t-Boc

chemistry, as described, for example, in Bodanszky, "The Principles of Peptide Synthesis", Hafner, Rees, Trost, Lehn, Schleyer, Zahradnik, Eds., Springer-Verlag, Berlin, 1984. The fragments so produced can be tested to identify those which can either to enhance or inhibit TNF-α transcription by interaction with the TNF-α promoter nucleotides CTCCC (–515 to –511), such as by microinjection assays. In an illustrative embodiment, LITAF peptide fragments containing a SQTWREPGAAGSPFHL peptide sequence (SEQ ID NO: 5) can be tested for their ability to enhance TNF-α transcription in a cell-based assay.

Kindly amend the specification at page 20, line 5, through page 21, line 4, as follows:

As discussed herein, it is important to be able to tightly regulate the expression of TNF-α by either up-regulation or down-regulation. The present invention features a method of identifying compounds that inhibit LITAF binding to a TNF-α promoter region that includes: a) incubating a mixture of a molecule containing a LSQTWREPGAAGSPFHL peptide sequence (SEQ ID NO: 3)(component 1); a molecule that includes a TNF-α promoter region (component 2), and the test compound (component 3); b) measuring the extent of binding of component 1 to component 2 in the absence of component 3; measuring the extent of binding of component 1 to component 2 in the presence of component 3); and determining the ratio of the binding measured in step c) to that measured in step b), wherein a decrease of binding in step c) relative to step b) indicates that the test compound inhibits the binding of LITAF to the TNF-α promoter region ion.

In contrast to methods described herein that relate primarily to the identification of compounds having the ability to interfere with LITAF binding to the TNF-α promoter (down-regulation of TNF-α), it is also possible to up-regulate TNF-α levels using a variety of methodologies. The present invention also features a method of identifying compounds that enhance LITAF binding to a TNF-α promoter region that includes: a) incubating a mixture of a molecule containing a LSQTWREPGAAGSPFHL peptide sequence (SEQ ID NO: 3)(component 1), wherein said molecule is not full-length LITAF; a molecule that includes the TNF-α promoter region (component 2), and the test compound (component 3); b) measuring the extent of binding of component 1 to component 2 in the absence of component 3; measuring the extent of binding of component 1 to component 2 in the presence of component 3); and determining the ratio of the binding measured in step c) to that measured in step b), wherein an increase of binding in step c) relative to step b) indicates that the test compound enhances the binding of LITAF to the TNF-α promoter region ion.

Kindly amend the specification at page 26, line 12, through page 30, line 25, as follows:

The series of hLITAF DNA fragments were generated by PCR with the following primer pairs and subcloned into the pGEX4T-1 vector (Pharmacia) (FIG. 1a): 1. Glutathione S-transferase (GST) hLITAF aa 1-75: 5'-CGGGATCCATGTCGGTTCCAGG ACCT-3' (SEQ ID NO: 6) and 5'-cggaattcggtaattggattgttatt-3' (SEQ ID NO: 7); 2. GST-hLITAF aa 1-151: 5'-CGGGATCCAT GTCGTTCCAGGACCT-3' (SEQ ID NO: 8) and 5'-

cggaattccagttgggacagtaatgg-3' (SEQ ID NO: 9); 3. GST-hLITAF aa 76-151: 5'-CGGGATCCGTGCAGACGGTCTACGTG-3' (SEQ ID NO: 10) and 5'-cggaattccagttgggacagtaatgg-3' (SEQ ID NO: 11); 4. GST-hLITAF aa 1-228: 5'-CGGGATCCATGTCGGTTCCAGGACCT-3' (SEQ ID NO: 12) and 5'-cgggatcctcagggtctca gggaggc-3' (SEQ ID NO: 13); 5. GST-hLITAF aa 76-228: 5'-CGGGATCCGTGCAGACGGTCTACGTG-3' (SEQ ID NO: 10) and 5'-cgggatcctcagggtctcagggaggc-3' (SEQ ID NO: 13); 6. GST-hLITAF aa 152-228: 5'-CGGGATCCCAGAGCTCT CCTGGGCAC-3' (SEQ ID NO: 14) and 5'-cgggatcctcagggtctcagggaggc-3' (SEQ ID NO: 13); 7. GST-hLITAF aa 152-228Δ181-195. The first in-frame mutant hLITAF DNA fragment was generated by PCR with primer pairs: 5'-CGGGATCCGGACCATTACTGTCCCAA-3' (SEQ ID NO: 15)(coordinates 435-456bp with BamHI) and 5'-ccaaaagaagacatggctggat gagaggtg-3' (SEQ ID NO: 16)(coordinates 621-531bp). The second hLITAF DNA fragment was generated by PCR with primer pairs: 5'-CATGTCTTCTTTTGGGGG-3' (SEQ ID NO: 17)(coordinates 609 to 624 bp) and 5'-cgggatcctcagggtct cagggaggc-3' (SEQ ID NO: 13)(coordinates 983-966 bp with EcoRI).

Both the first and second DNA fragments were purified and diluted as template to 1 ng/reaction, and amplified by PCR with primer pairs: 5'-CGGGATCCGGACCATTACTG TCCCAA-3' (SEQ ID NO: 15)(coordinates 435-456bp with BamHI) and 5'-cgggatcctcagggtctcagggaggc-3' (SEQ ID NO: 13)(coordinates 983-966 bp with EcoRI). Finally, the in-frame hLITAF mutant DNA fragment was inserted into the pGEX4T-1 vector; 8. GST-hLITAF aa 152-228Δ164-180. The first in-frame mutant hLITAF DNA fragment was generated by PCR with primer pairs: 5'-CGGGATCCGG ACCATTACTGTCCCAA-3' (SEQ ID NO:

15)(coordinates 435-456bp with BamHI) and 5'-tccaccaggcgtga atcctacaaacgcttg-3' (SEQ ID NO: 18)(coordinates 564 to 477bp). The second hLITAF DNA fragment was generated by PCR with primer pairs: 5'-TTCACGCCTGGTGGAGGT-3' (SEQ ID NO: 19)(coordinates 552 to 570 bp) and 5'-cgggatcctcagggtctcagggaggc-3' (SEQ ID NO: 13)(coordinates 983-966 bp with EcoRI). Both first and second DNA fragments above were purified and diluted as template to 1 ng/reaction, and amplified by PCR with 5' and 3' primers, 5'-CGGGATCCGGACCATTACTGTCCCAA-3' (SEQ ID NO: 15)(coordinates 435-456bp with BamHI) and 5'-cgggatcctcagggtctcagggaggc-3' (SEQ ID NO: 13)(coordinates 983-966 bp with EcoRI). Finally, the in-frame mutant DNA fragment was inserted into the vector.

Subcloning the hLITAF DNA fragments into a pGL3-Basic Expression Construct

The following series of hTNF-α promoter DNA fragments were subcloned (FIG. 1b) into the pGL3-Basic vector, which has a promoterless and enhancerless luciferase reporter gene, available from Pharmacia: 1. wtTNFP (–991 to 1) was generated by PCR with primer pairs: 5'-AGCTCCTGG GAGATATGGCCAC-3' (SEQ ID NO: 20) and 5'-gggtgtgccaacaactgccttt-3' (SEQ ID NO: 21). 2. mtTNFP1 (–991 to 1Δ–515 to –511). The first in-frame mutant hTNF-α promoter was generated by PCR with primer pairs, 5'- AGCTCCTGGGAGATATGGCCAC-3' (SEQ ID NO: 20) and 5'-tgcgaaggagctgggggctt (SEQ ID NO: 22). The second mutant DNA was generated by PCR with primer pairs, 5'-CCTTCGCAGGGACCCAAACACAGGCCTCA-3' (SEQ ID NO: 23) and 5'-gggtgtgccaacaactgccttt-3' (SEQ ID NO: 21). Both first and second DNA fragments above were purified and diluted as template to 1 ng/reaction and finally amplified by PCR with primer pairs, 5'-AGCTCCTGGGAGATATGGCCAC-3' (SEQ ID NO: 20) and 5'-

gggtgtgccaacaactgccttt-3' (SEQ ID NO: 21). 3. mtTNFP2 (–550 to –487 plus TATA Box) was generated by annealing with primer pairs: 5'-AGGCCTCAAGCCT GCCACCAAGCCCCCAGCTCCTTCTCCCCGCAGGGACCCAAACACAGGCCTCATATA AAGGCAGTTGTTGGCACACCC-3' (SEQ ID NO: 24) and 5'-gggtgtgccaacaactgcctttatatgaggcctgtgtttgggtccctg cggggagaaggagctgggggcttggtggcaggc ttgaggcct-3' (SEQ ID NO: 25). 4. mtTNFP3 (–550 to –487Δ–515 to –511 plus TATA Box) was generated by annealing with primer pairs: 5'-AGGCCTCAAGCCTGCC ACCAAGCCCCCAGCTCCTTCGCAGGGACCCAAACACAGGCCTCATATAAAGGCAGT TGTTGGCACACCC-3' (SEQ ID NO: 26) and 5'-gggtgtgccaacaactgcctttatatgaggcctgtgtttgggtccctgcgaaggagctgggg gcttggtggcaggcttgagg cct-3' (SEQ ID NO: 27).

Purification of GST-hLITAF fusion protein

GST-hLITAF recombinant plasmids were transformed into competent BL21 cells. LBA medium (2 ml) was inoculated with a single colony of the appropriate transformant for culture at 37° C overnight. This 2 ml culture was then transferred to 100 ml of 2x YTA broth plus ampicillin (100 μg/ml) and grown at 30° C with shaking until the absorbance at 600 nm reached 0.6, at which time IPTG was added to a final concentration of 0.1 mM. The culture was incubated for an additional 2-6 hrs, then subjected to centrifugation at 3,000 x g for 10 min at 4° C. The cells were washed with PBS and completely suspended in 2 ml of ice-cold PBS, then lysed by brief sonication for 10 sec (output 20, Branson Sonifier 450,), then centrifuged twice at 5,000 x g for 10 min at 4° C. The supernatant was transferred to a fresh container, to which was

added 100 µl of Glutathione-Sepharose 4B beads (Pharmacia), and the mixture was rocked for 30 min at 4° C, then washed three times with PBS. Protein samples were run in 10 % SDS-PAGE.

DNase I Footprinting

The protein-DNA binding site was analyzed by the DNase I footprinting method (Galas and Schmitz, Nucleic Acids Res. 5:3157-3170, 1978) with some modifications. Two oligonucleotides were synthesized. The first one was designed as a template, with a Hind III site at the 5' end. For the reverse orientation, nucleotides from –487 to –550 bp in the hTNF-α promoter were represented (5'-TGAGGCCTGTGTTTGGGTCCCTGCGGGGAGAAGGAGCTGGGGGCTTGGTGGCAGG CTTGAGGCCT-3'; SEQ ID NO: 28). The second one was designed as a primer from –550 to –535 bp in the hTNF-α promoter, 5'-aggcctcaagcctgcc-3' (SEQ ID NO: 29). Template (0.5 µg) and 0.1 µg primer were mixed and incubated at 37° C for 1 hr, then 2 µl 2.5mM 4dNTP mix, 5 µl 10x Klenow fragment buffer, 5 units Klenow fragment (Invitrogen), and water to 50 µl were added, and incubated at 37° C for 30 min. The DNA was purified, then precipitated with ethanol. After centrifugation, the DNA pellet was suspended in 10 µl TE buffer. DNA (0.5 µg) was labeled with γ-[^{32}P]ATP using T4 polynucleotide kinase (Promega) and then digested by HindIII as previously described (Donis-Keller, H., Nucl. Acids Res. 8:3133, 1980). Labeled DNA was purified using a G-25 Sephadex column (Boehringer) and precipitated with ethanol. After centrifugation, the DNA pellet was suspended in 10 µl water. The γ-[^{32}P]ATP-labeled DNA was then mixed with 25 µl binding buffer (Promega), 0.1 µg GST-hLITAF fusion protein (GST fusion protein alone as control), and nuclease-free water (Promega) to 50 µl, incubated on ice for 30 min, to which 50 µl pre-warmed Ca^2 / Mg^2 solution at RT was added and incubated for

one min, then 3 µl DNase I (Promega) was added, mixed gently, incubated for an additional 5 min, followed by reaction termination. The reaction mixture was treated with phenol and precipitated with ethanol. After centrifugation, the DNA pellet was suspended in 5 µl of TE buffer. The sample was applied to a 6% polyacrylamide sequencing gel (Invitrogen)

Electrophoresis Mobility Shift Assay

A reaction mixture containing 0.1 µg GST-hLITAF fusion protein, 1 µl radiolabeled ($1x10^5$ cpm /µl) double stranded DNA oligonucleotide (2 pmol), 3 µg poly(dI/dC), 5 µg bovine serum albumin, 4 µl gel shift binding 5x buffer (Promega), and nuclease-free water to 20 µl, was incubated at RT for 30 min prior to electrophoresis on non-denaturing 6% polyacrylamide gels in Tris-borate-EDTA buffer (90 mM Tris-borate, 2 mM EDTA HEPES [pH8.0]).

Peptides

The following synthetic peptides were supplied by Lofstrand Labs Ltd (Gaithersburg, MD): Peptide A consisted of the sequence (SYYTQPAPIPNNNPIT VQTVY; SEQ ID NO: 30) from the hLITAF aa 60-80; peptide B consisted of the sequence (SQTWREPGAAGSPFHL; SEQ ID NO: 5) from aa 165 to 180; and peptide C consisted of the sequence (LSSSFTPGGGSALVVS; SEQ ID NO: 31) from aa 180 to 195 (FIG.1, 4, and 5). Hemagglutinin antigenic peptide (HA) served as control peptide and consisted of the sequence (YPYDVPDYASL; SEQ ID NO: 32). All peptides were solubilized in DMSO and delivered into THP-1 cells by Chariot kit (Chariot Motif, 1914 Palomar Oaks Way, Suite 150, Carlsbad, CA

92008) for reporter assays as described in references Horng et al., Nature Immunology 2:835-841, 2001; Morris et al., Nat. Biotechnol. 19:1173-6, 2001.

Kindly amend the specification at page 35, line 12, through page 36, line 13, as follows:

As described herein, the region within human LITAF (hLITAF) that specifically mediates DNA binding resides in the sequence corresponding to hLITAF aa 164-180 (i.e., peptide sequence SQTWREPGAAGSPFHL; SEQ ID NO: 5), and LITAF protein fragments corresponding to that area were determined to be sufficient to bind and activate the TNF-α promoter. As also described herein, the sequence motif CTCCC (–515 to –511), within the TNF-α promoter, binds to hLITAF aa 164-180. Several studies have shown that a known tumor suppressor gene, p53, participates in inducing apoptosis in response to a variety of stress stimuli, including ionizing radiation, cytotoxic agents, oxidative stress, and LPS (see Kinzler and Volgolstein, Nature 379:19-20, 1996; Ko and Prives, Genes Dev. 10:1054-1072, 1996; Levine, A.J., Cell 88:323-331, 1997; and Munshi et al., J. Immunol. 168:5860-5866, 2002). Normally p53 functions as a transcription factor that regulates DNA repair, cell proliferation, and cell death. It has been shown to upregulate the apoptosis inducer BAX (Miyashita and Reed, Cell 80:293-299, 1995) and to down-regulate a competing cell survival signal, Bcl-2 (Adams and Cory, Science 281:1322-1326, 1998). The cyclin-dependent kinase inhibitor p21, involved in cell growth arrest, is also regulated by p53 (Brugoarolas et al, Nature 377:552-557, 1995; el-Deiry et al, Cell 75:817-825, 1993; Vousden and Lu, Nat. Rev. Cancer 2:594-604, 2002).

Several studies have also indicated that the expression of p53 could be detected after prolonged (24 hrs), but not brief (6 hrs), treatment with LPS (Munshi et al., J. Immunol. 168:5860-5866, 2002; Xaus et al., Blood 95:3823-3831, 2000). However, it is also known that LITAF is induced after only 2-4 hrs of treatment with LPS (Myokai et al., Proc. Natl. Acad. Sci. USA 96: 4518-4523 (1999); Tang et al., Proc. Natl. Acad. Sci. USA, 100:4096-101, 2003) and that LITAF mRNA is markedly increased in p53-expressing cells (Polyak et al., Nature 389:300-305, 1997). However, the signaling pathway(s) whereby p53 induces apoptosis through TNF-α in response to LPS remain unclear. The following experiments described below were performed to determine the role of p53 in the expression of LITAF and what effect LPS had on LITAF expression.

Kindly amend the specification at page 37, lines 8-12, as follows:

MSVPGPYQAATGPSSAPSAPPSYEETVAVNSYYPTPPAPMPGPTTGLVTGPDGKG MNPPSYYTQPAPIPNNNPITVQTVYVQHPITFLDRPIQMCCPSCNKMIVSQLSYNAGALT WLSCGSLCLLGVHSGLLLHPLLRGCPAGRGPLLSQLQSSPGHLQAFVGLSQTWREPGAA GSPFHLSSSFTPGGGSALVVSPLQGAHLHVFFWGEYVAKLTNLQTPEIAAWSRA. (SEQ ID NO: 1)